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SPECIAL SECTION:
GRA N_2O CHAMBER METHODOLOGY GUIDELINES

Global Research Alliance N_2O chamber methodology guidelines: Recommendations for air sample collection, storage, and analysis

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Abstract

Certain aspects in the collection, handling, storage, and subsequent analysis of discrete air samples from non-steady-state flux chambers are critical to generating accurate and unbiased estimates of nitrous oxide (N_2O) fluxes. The focus of this paper is on air sample collection and storage in small vials (<12 ml) primarily for gas chromatography (GC) analysis. Sample integrity is assured through following simple procedures including storage under pressure and analysis within a few months of collection. Concurrent storage of standards in an identical manner to samples is recommended and allows the storage period to be reliably extended. In the laboratory, an autosampler is typically used in batch analysis of ~200 sequentially analyzed samples by GC with an electron capture detector (ECD). Some comparisons are given between GC and alternatives including optical N_2O detectors that are increasingly being used for high-precision N_2O measurement. The importance of calibration and traceability of gas standards is discussed, where high-quality standards ensure the most accurate assessment of N_2O concentration and comparability between laboratories. The calibration allows a consistent and best estimate of flux to be derived.

1 | INTRODUCTION

This paper describes the collection and storage of air samples, and the method of analysis to determine their nitrous oxide (N_2O) concentrations as sets of discrete samples collected from manually operated static chambers for determining soil N_2O emission (exchange) fluxes. The primary focus is on use of gas chromatography (GC) with an electron capture detector (ECD) for gas analysis, although we discuss alternative detectors that are increasingly being used. For details of the GC methods, we refer the reader to Mosier and Mack (1980). Methods described in this paper are based on those used at New Zealand's National Centre for N_2O Measurement in Lincoln, New Zealand (NZ-NCNM), and the National Institute of Water and Atmospheric Research (NIWA) Gas Laboratory in Wellington,

Abbreviations: AFBI, Agri-Food and Biosciences Institute; CCL, Central Calibration Laboratory; CFC, chlorofluorocarbon; ECD, electron capture detector; FID, flame ionization detector; FTIR, Fourier-transform infrared; GC, gas chromatography; MS, mass spectrometry; NIWA, National Institute of Water and Atmospheric Research; NZ-NCNM, New Zealand's National Centre for Nitrous Oxide Measurement; QCL, quantum cascade laser; WMO, World Meteorological Organization.

New Zealand, and build on the experience of others. Where appropriate, we introduce the underlying principles for guidance in quantifying analytical data repeatability and reproducibility.

Although automated chamber methodologies have evolved to achieve reliable measurements, manual sampling techniques are still widely used and continue to contribute to national reference datasets that underpin the estimation of emission factors used in national greenhouse gas inventory reporting. It is therefore of high importance that measurement methods are standardized as much as possible. Without being highly prescriptive, in this paper, we summarize the main aspects to consider in sample collection, storage, and laboratory determination of N_2O in samples with the associated calibration using gas standards. We present minimum requirements to ensure that resultant emission estimates are accurate. Several alternatives to GC–ECD detection are discussed.

The background to and development of the N_2O chamber methodology guidelines is described in the introductory paper by de Klein et al. (2020). This paper on sampling and analysis draws on previous work by the Global Research Alliance (GRA, https://globalresearchalliance.org/) and guidelines for chambers of other networks, such as the USDA (Parkin & Venterea, 2010) and Integrated Carbon Observing System (ICOS, Pavelka et al., 2018). Related papers in this special section discuss static chamber design (Clough et al., 2020), automated systems (Grace et al., 2020), and flux calculation methods (Venterea et al., 2020).

Core Ideas

- Inert pre-evacuated septum-sealed vials are recommended for air sample collection from chambers.
- Adequate flushing and overpressuring gas in the vial helps to maintain sample integrity.
- Short storage times and concurrent storage of standards is important for quality assurance.
- Standard gas chromatography methods are summarized along with alternative detectors.
- Traceable gas standards enable accurate and intercomparable gas concentration estimates.

2 | GAS MEASUREMENT CONVENTION

We recommend "concentration" units for N_2O that are expressed as the dry air mole fraction in units of moles of N_2O per mole of dry air (nmol mol^{-1}, the ratio of the number of N_2O molecules per 10^9 molecules of dry air is often abbreviated as parts per billion; μmol mol^{-1}, the ratio of the number of N_2O molecules per 10^6 molecules of dry air is often abbreviated as parts per million). This is an unambiguous ratio quantity not requiring any implicit assumptions of obeying ideal gas laws. In this paper, we use the term "concentration" to be synonymous with the "dry air mole fraction" because "concentration" is more commonly used in the flux chamber measuring community. Furthermore, at ambient temperatures and pressures, N_2O is behaving effectively as an ideal gas and the standardized volumetric fraction (i.e., nl N_2O L^{-1} dry air) can be regarded as equivalent to the mole fraction.

The potential impact of water vapor is an important consideration in concentration measurement in atmospheric samples, and we therefore follow the best practice to measure and express values relative to dry air (drying the sample to remove the measurement variability due to varying amounts of water vapor molecules). The best practice is to dry the gas samples collected from static chambers at the time of collection. Supplemental Section S1 discusses the water vapor correction in more detail. For further background, readers are also directed to Global Atmosphere Watch (GAW) Report 185 (WMO, 2009), which provides comprehensive guidance on air sample analysis, including quality control and assurance.

3 | COLLECTION AND STORAGE OF AIR SAMPLES

3.1 | Gas sample collection containers: Vial preparation

Air sample vials (containers) need to be leak proof, clean, and made of materials that do not react with N_2O. Although good-quality, gastight syringes may be suitable for short-term storage of samples, they are an expensive option. We recommend septum-sealed containers that are evacuated (<100 Pa) prior to sampling, which means the container must be able to withstand this process, and maintain the vacuum, prior to sampling. The container should remain gastight afterwards to prevent sample loss during storage before analysis. Rochette and Bertrand (2003) discuss issues and report the results of a comparison of polypropylene syringes and glass vials. Glass vials (e.g., Exetainer, Labco) are now commonly used as air sample containers, and procedures have been developed for their use. Although different sizes are available, 6- and 12-ml septum-capped glass vials are most commonly deployed with air sample volumes as small as 1 ml being removed for analysis, as dictated by the flushing requirements of the analytical system to ensure that sample concentration

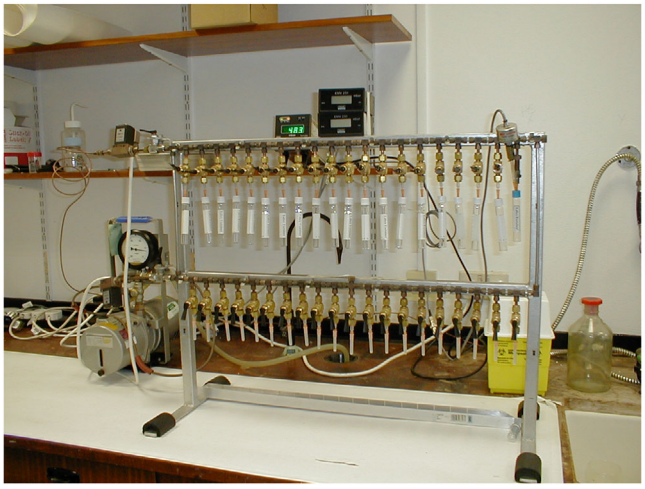

FIGURE 1 A (12-ml) vial evacuation system, including the vacuum pump, vacuum gauge, manifold, valves, and needles for penetrating septa, as shown in the upper half of the manifold. The system shown is that used at the Agri-Food and Biosciences Institute in Northern Ireland (AFBI)

is unaltered in transfer to the detector (Hedley, Saggar, & Tate, 2006; Rassmussen, Krasnec, & Pierotti, 1976).

These glass vials have screw-on plastic caps with butyl-rubber septa. Experience shows that gastightness is achieved when the cap is screwed on "finger tight," followed by another quarter turn (Pavelka et al., 2018). Different septa are available. At the NZ-NCNM, 3-mm-thick, gray-colored butyl-rubber septa have been used, whereas at the Agri-Food and Biosciences Institute in Northern Ireland (AFBI), the septa consist of a double-wadded "sandwich" of 3-mm-thick butyl-rubber septum and a polytetrafluoroethylene (PTFE)–silicone septum (see also Rochette & Bertrand, 2003). These chosen septa have been checked for nonreactivity to N_2O over expected storage periods. Laughlin and Stevens (2003) showed that significant N_2O concentration reductions may occur after a year of storage in butyl-rubber-capped vials and suggest a compensation method where calibration standard gases are stored in a similar manner along with the samples.

Pre-evacuated vials have been shown to contain variable amounts of residual gas that can be a source of N_2O contamination (Sturm et al., 2015). As an alternative to evacuation, de Klein, Barton, Sherlock, Li, and Littlejohn (2003) described an equally effective flushing procedure, in which, before sample collection, 25 ml of chamber head space air was flushed backwards and forwards through a 6-ml septum-capped glass vial container four times. If vials are to be evacuated, a system begins with a vacuum pump (Figure 1). As an example, at NZ-NCNM, a dry pump is used, isolating the bearings and their hydrocarbon lubricant from the vacuum space (Model XDS5, 5 for a peak pumping speed of 5 m^3 h^{-1}, Edwards). The pump is connected by tubing to a vacuum gauge and by further tubing to a manifold. Depending on the number of samples, a number of manifolds may be required, and at NZ-NCNM, three 14-port manifolds (Model WMF6000, SJ4 Manufacturing Services) have been connected in series to the pump. Each port has a two-way valve and a 25-gauge × 16-mm (5/8) needle (Becton, Dickinson and Company). This stainless needle has 0.50- and 0.24-mm nominal outside and inside diameters, respectively.

The angled, sharpened tip of this needle is called a "regular bevel" by the manufacturer. The needle is inserted through each rubber septum into the vial, and the sharpened tip with regular bevel angle has been designed to minimize the required force and drag. The bevel should not be longer than the thickness of the septum; otherwise, air will enter when the vial is removed from the evacuation manifold. Common-sense precautions should be taken in working with needles to avoid personal injury. Needles should be inspected regularly, because a damaged or worn bevel may cause septum damage and thereby leakage. The evacuation rate becomes very slow after 3 or 4 min (Rochette & Bertrand, 2008), and at NZ-NCNM, evacuation is done for 5 min. Alternatively, at AFBI, vials are evacuated to <100 Pa from a connected, pressurized tank, through a combination of evacuation and purging with helium, an inert gas. Rochette and Bertrand (2008) noted that the smaller helium molecule allowed more rapid and complete purging of vials.

The vacuum in a vial can be tested using a two-way needle, with one end inserted into a container filled with water and the other penetrating a septum, and "sipping" water into the vial before weighing it for comparison with the "empty" weight (Rochette & Bertrand, 2008). Rochette and Bertrand (2003) showed that vials with double-wadded septa could be evacuated up to 63 d prior to use, and that septa could be reused up to seven times. As shown below, each use may involve piercing a septum up to four times for (a) vial evacuation, (b) sample collection (including overpressurizing the vial), (c) sample equilibration to atmospheric pressure (for systems where the vial is part of the injection loop), and (d) sample analysis by the GC system. Results of the septa-piercing experiments reported by Glatzel and Well (2008) corroborated the earlier recommendation of Rochette and Bertrand (2003) regarding septa reuse with a significant advantage and lower leakage rate where smaller canula (0.45 mm) are used.

3.2 | Sample collection

We recommend that an air sample is collected using a gastight polypropylene or glass syringe, connected to a gastight valve and needle (same specifications as above).

To collect a sample, the syringe with shut-off valve is connected to the gas sampling port of the static chamber taking air from the chamber headspace. The syringe plunger is then pumped a few times to flush the syringe and any dead volume (de Klein et al., 2003). A sample can be transferred directly into an evacuated vial. The sample volume should be ~10 and ~20 ml for 6- and 12-ml vials, respectively, thereby overpressurizing the vial to minimize the incursion of ambient air during storage. In systems where the sample overpressure drives flushing and filling of a sample injection loop in the analytical system, use of larger (12-ml) vials provides the analyst with more sample and the possibility of rerunning vials with erroneous values or high concentrations. The initial movement of the syringe plunger when the needle penetrates the septum is a useful visual indication that the vial was pre-evacuated. If the syringe plunger does not move spontaneously, it may indicate that the vial was either not evacuated or not leak tight. As discussed by others (Collier, Ruark, Oates, Jokela, & Dell, 2014), air temperature and pressure should be recorded for accurate conversion of gas concentration to mass flux, and other ancillary measures such as soil temperature and soil moisture content at each location and daily rainfall, soil bulk density, and soil nitrate and ammonium concentrations may be required.

3.3 | Storage

Once collected, the air sample must be stored until laboratory analysis, and the container should prevent sample loss and maintain integrity of the sample (i.e. not cause significant concentration change prior to analysis). For samples with an N_2O concentration of 10 μmol mol^{-1} and storage periods of 14 and 126 d, between 92 and 98% of the original N_2O concentration could be recovered from containers using butyl-rubber and doubled-wadded septa, respectively (Rochette & Bertrand, 2003). For optimal recovery Faust and Liebig (2018) found negligible change in Exetainer vials with chloro-butyl septa when analyzed within 28 d after storage over a wide (−10 to 25 °C) temperature range. Further they found N_2O concentrations generally within 95% of original value after 84 d. Using butyl-rubber septa over longer periods, for samples with an N_2O concentration of 1,000 nmol mol^{-1} and storage period of 365 d, 90% of the original N_2O concentration was recovered, and the decrease in N_2O concentration over time was linear (unpublished results from the NZ-NCNM laboratory). During storage, the external ambient N_2O concentration is ~300 nmol mol^{-1}, and the leakage rate from the vials will be proportional to the concentration gradient, according to diffusion theory (Laughlin & Stevens, 2003).

With the likelihood of variation in vials and other factors between laboratories, we recommend that every laboratory do a storage test and repeat it whenever changes to the vial types, caps, or evacuation system have been made. Storage tests should even be done for short storage periods of 1 wk. They should be done weekly in the first months and then monthly for 1 yr, and a range of standard gases should be used.

3.4 | Sampling recommendations

The following recommendations are therefore minimum requirements for sample collection and storage to guarantee integrity of stored samples:

- Use proven vial and septa types.
- Minimize vial storage period to preferably <1 mo.
- Overpressurize vials (1.6 times vial volume) to ensure sample integrity.
- Include sample storage tests in gas analysis batches by including storage of a range of standard gases.
- Pay close attention to quality checking of sample integrity when storage is longer than 6 mo.
- Reject samples where pressure has been lost.

4 | ANALYTICAL SYSTEMS AND DETECTOR OPTIONS

An increasing variety of N_2O detectors are available for determining concentrations, either directly in the field in online automatic systems (Grace et al., 2020) or from laboratory analysis of chamber headspace samples stored as described above. A third approach, directed towards maximizing data from campaigns in remote or hill country regions, has been to use manual chambers with a portable field analyzer (Debouk, Altimir, & Sebastià, 2018). This approach is likely to become more common when more portable high-precision N_2O analyzers become available.

Table 1 shows commonly used detectors. We also refer readers to reviews by Butterbach-Bahl, Sander, Pelster, and Díaz-Pinés (2016), Rapson and Dacres (2014), and Hensen, Skiba, and Famulari (2013). We provide typical precision and accuracy figures for the GC methods and manufacturer-quoted precision of alternative detectors. High accuracy is achievable with high-precision systems but will require careful traceable calibration and optimization of instrument operation.

TABLE 1 Summary of detector systems for analysis of N_2O from chamber systems after Rapson and Dacres (2014) and Lebegue et al. (2016)

System/detector[a]	Limit of detection	Precision range	Precision (1–σ)[b]	Typical sample introduction	Sample size/flow-rate notes
	nmol mol^{-1}		nmol mol^{-1}		
GC–ECD (Ref 1)	<30	Mid	0.18–0.4	Vial injection	0.35–1 ml
GC–multi-gas (Ref 2)	7.4	Mid	0.74, accuracy: 5%	Vial injection	1 mL loop
GC–MS (Ref 3)	20	Low	±3%, accuracy: 5%	Vial injection	5 µl injection; 2.0 ml min^{-1} carrier
Photoacoustic spectroscopy (Refs 4A, 4B, 4C)	>7	Low	1.5–5%, accuracy: <15%	Continuous, online to chamber	300 ml min^{-1} (cross-sensitivity interferences corrected for H_2O, CO_2)
Gas filter correlation (Ref 5)	10	Low	1.5 (10 min)	Online	750 ml min^{-1}
Difference frequency generation[c]		Mid	0.16 (1 min), 0.11 (10 min), 0.21 (LT[d])	Online	300 ml min^{-1}
Closed-cell FTIR (multi-gas) (Ref 6)	<1	High	0.15 (I min), 0.06 (10 min), 0.07 (LT)	Online	3.5 L cell better with larger volume recirculation
Tuneable diode laser (lead salt) (Refs 7A, 7B)	1	High	<1 (5 s), 0.03 in 20 min	Online	~1,000 ml min^{-1} with critical orifice
Cavity ring down spectroscopy (CRDS)[e]	1	High	0.06 (1 min), 0.03 (10 min), 0.07 (LT)	Online	100 ml min^{-1}
Quantum cascade laser (QC-TLDAS)[f]	0.03–1	High	0.08 (1 min), 0.07 (10 min), 0.14 (LT)	Online	1,000 ml min^{-1}
Off-axis–integrated cavity output spectroscopy (OA–ICOS)[g]	<1	High	0.07 (1 min), 0.06 (10 min), 0.3 (LT)	Online	300 ml min^{-1}

[a]GC, gas chromatography; ECD, electron capture detector; MS, mass spectrometry; FTIR, Fourier-transform infrared. Ref 1: Hamilton and Lewis (2006); Ref 2: Hedley et al. (2006); Ref 3: Ekeberg et al. (2004); Ref 4A: Kang et al. (2014); Ref 4B: Iqbal et al. (2013); Ref 4C: Tirol-Padre et al. (2014); Ref 5: Fassbinder, Schultz, Baker, & Griffis (2013); Ref 6: Griffith et al. (2012); Ref 7A McMillan et al. (2014); Ref 7B Officer et al. (2015). [b]Including detector performance evaluation by Lebegue et al. (2016). [c]e.g., Thermo IRIS4600. [d]LT, long-term repeatability assessment, a measure of stability over several days (Lebegue et al., 2016). [e]e.g., Picarro G5310. [f]Aerodyne QCL Mini. [g]Los Gatos N_2O.

5 | SAMPLE HANDLING AND ANALYSIS BY GC

5.1 | Sample gas drying

Sample gases are often collected into vials and analyzed without in-line drying of the sample. Compared with the calibration gases, which are dry, there is an error introduced with samples as a result of the water vapor dilution. Further, there is likely to be an increasing water vapor dilution over time due to the water vapor flux into the soil chamber during the period of closure. An estimate of the magnitude of the error and the correction is discussed in Supplemental Section S3 and by Parkin and Venterea (2010). Estimated corrections of ~3% are likely to be a maximum because some drying is likely to occur to container surfaces and in the swept part of the analytical system. Removal of water vapor is possible as described by Mosier and Mack (1980) with a miniature in-line magnesium perchlorate trap. Testing with such a system is needed to confirm sample size is adequate for flushing the inlet and there are no carryover or artifacts introduced into sample determination. In online multi-gas systems, it may be possible to measure water vapor directly with a measurement frequency of a few seconds, as discussed by LI-COR Biosciences for their soil carbon dioxide (CO_2) flux system (https://www.licor.com/env/support/LI-8100A/topics/deriving-the-flux-equation.html).

5.2 | Sample introduction in GC systems

Injected sample volumes from vial samples are typically in the range of 1–6 ml. At the NZ-NCNM, the glass vial essentially takes the place of the fixed-volume sample loop normally used for gas analysis, in more conventional GC injection procedures. Gas flows through the vial using a double concentric bore needle. Just prior to GC analysis, the sample pressure is equilibrated to ambient atmospheric pressure, so the sample will have the same volume as the internal volume of the vial. This is done using a simple, inexpensive, double-ended needle device (i.e., BD Vacutainer Eclipse blood collection needle), similar to that described earlier, but here with an upwards-facing needle that can penetrate a septum cap, with the other end of the needle inserted into a container filled with water.

When the septum cap of an overpressured vial is penetrated, the release of pressure produces bubbles in the water. When the bubbling stops, typically after 2 or 3 s, it is a visual indication that ambient atmospheric pressure has been reached. An alternative sample injection procedure exploits the overpressure in the vial to flush sample gas through a fixed-volume gas sample loop (typically 1–5 ml), which forms part of a gas-sampling valve (Hedley et al., 2006). Where larger volume vials (12 ml) are used, there can be adequate gas for two consecutive injections. In the case of high concentration (over-range) samples, all vials have the possibility of being rerun following a repressurizing addition of a known volume of zero air (i.e., purified whole air or a N_2/O_2 synthetic mixture of atmospheric proportions with water vapor, N_2O, trace hydrocarbon, and other reactive gases removed).

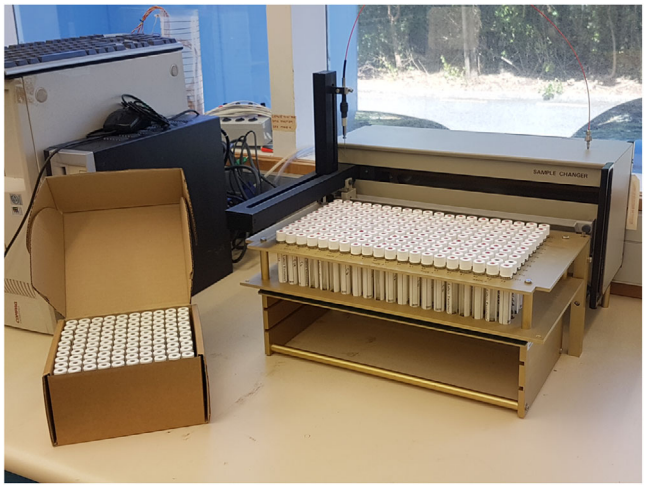

FIGURE 2 Vial autosampler sample changer system loaded with 220 Exetainers at the National Institute of Water and Atmospheric Research (NIWA) Gas Laboratory (12 ml)

The GC injection valve is switched by an electrical or pneumatic actuator between flushing carrier gas through the loop and injecting the sample onto the GC column. We provide details here of the automated analytical procedure used at the NZ-NCNM. Details of other automated procedures are described by Zheng et al. (2008) and the references contained therein. Large numbers of stored samples can be programmed to run using an autosampler system feeding sequential samples automatically for GC analysis. There are a number of commercial samplers capable of handling >200 vials, such as Gilson 222XL (Gilson) as shown in Figure 2 and used by Hedley et al. (2006) and other units by PAL (CTC Analytics), Bandolero, (xyzTecg.com), and even open-source hardware designs (Carvalho & Murray, 2018).

At the NZ-NCNM, the automated (Gilson 222XL) vial sampler is also used. The sampling arm on the handler is fitted with a double-cavity concentric needle (Stevens, Laughlin, Atkins, & Prosser, 1993). These needles were originally designed for injection of discrete gas samples into isotope ratio mass spectrometers and are supplied through Sercon. Needle movement is controlled by the handler's software, synchronized with a valve-switching

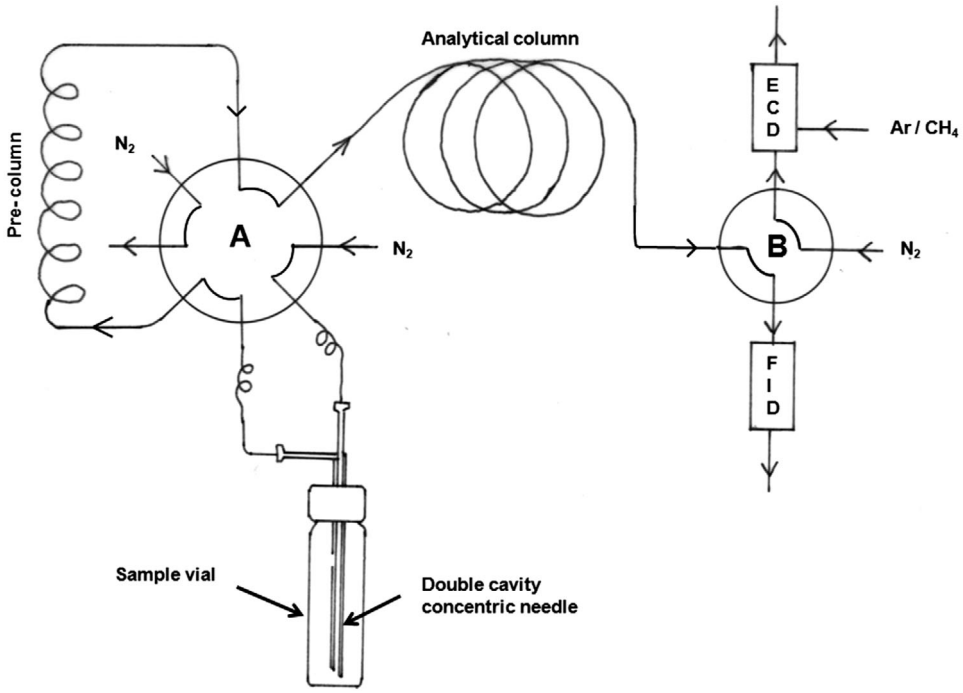

FIGURE 3 Simplified plumbing diagram, showing the gas sampling valves in the inject mode as described in the text. The system shown is that used at New Zealand's National Centre for Nitrous Oxide Measurement (NZ-NCNM) and shows a second detector (flame ionization detector [FID]). Only the electron capture detector (ECD) is required for N_2O determination

sequence using the GC software control and acquisition system (Peak Simple, SRI Instruments).

It is good practice to interspace samples with calibration standards. A typical analysis "run" of 220 Exetainers can be constructed with an initial block of (~7 to 10) standards and ambient reference gas followed by blocks of 13–15 chamber air samples separated by two ambient reference samples. Standards for the purpose of GC system calibration should span beyond the range of unknown N_2O concentrations. These standards are taken from pressurized gas cylinders and transferred into vials. The run is completed by a second batch of N_2O standards. Choice of standards is described in detail below in Section 6.2. The analysis of some groups of replicate samples collected in the field is recommended for assessing overall experimental uncertainty.

Sample injection, pre-column "backflush" (see below) and sample passage through the main analytical column to the ECD is controlled by two 10-port gas sampling valves, one of which is located at the head of the pre-column, whereas the other is located immediately prior to the ECD. The first 10-port valve (Valve A) is actually configured as an eight-port valve, whereas the second 10-port valve (Valve B) is configured to operate as a simple four-port switch, whose main function is to direct the flow from the main analytical column either into, or away from, the ECD (Figure 3). This is essentially the same design as first used by Mosier and Mack (1980).

5.3 | Analysis by GC with an electron capture detector

The ECD was first reported by Lovelock and Lipsky (1960). It detects electronegative compounds, including N_2O. The detector contains a sealed radioactive source of beta particles (e.g., ^{63}Ni decaying at 185 MBq [5 mCi], SRI Instruments). A carrier gas mixture comprising 10% methane in argon (known as P10) in the presence of the ^{63}Ni β particle source produces low-energy free electrons, which induce a steady background current in the collector plates of the detector. When the N_2O molecules exit the column into this free-electron-rich environment, they have a tendency to absorb or capture these free electrons. The detector electronics are designed to maintain a constant current (~1 nA) through the electron cloud and pulse at a faster rate to compensate for the decreased number of free electrons (Maggs, Joynes, Davies, & Lovelock, 1971; Wentworth, Chen, & Lovelock, 1966; Wentworth & Freeman, 1973).

The detector pulse rate is monitored and integrated to calculate the N_2O concentration, according to calibration of the GC system described below. Although the original Mosier and Mack (1980) GC system (Figure 3) used an Ar/CH_4 mixture as the carrier gas, many researchers now use less expensive N_2 together with the optional addition of some P10 as a makeup gas to improve detector response. Although the ECD detector response is nonlinear, the

TABLE 2 Examples of two laboratory gas chromatography (GC) configurations

Laboratory	NZ-NCNM[a]	NIWA[b] Gas Laboratory
Gas chromatograph	SRI Instruments/ECD[c]	Agilent 6890A/ECD
ECD temperature, °C	310	390
Carrier gas	Purified N_2 with addition of P10 (10% CH_4 in Ar) makeup gas	P10 (10% CH_4 in Ar)
Carrier flow rate, ml min^{-1}	40	30
GC 6 port valve		Valco A4C10UWE
Sample loop	6-ml vial	5 ml
Pre-column	3-mm (1/8th-inch) HayeSep-D (1 m) 125–149 μm mesh	3.2-mm (1/8th-inch) Porapak-Q (1.5 m) (80–100 mesh)
Main column	3-mm (1/8th-inch) HayeSep-D (4 m) 125–149 μm mesh	3.2-mm (1/8th-inch) Porapak-Q (3 m) (80–100 mesh)
Oven temperature, °C	40	63
N_2O elution, min	8	5

[a]NZ-NCNM, New Zealand's National Centre for Nitrous Oxide Measurement. [b]NIWA, National Institute of Water and Atmospheric Research. [c]ECD, electron capture detector.

signal is improved with the makeup gas. Dinitrogen is not a particularly effective detection medium for N_2O when using a ^{63}Ni ECD, but by introducing the more ionizable P10 makeup gas separately into the ECD, the N_2O peak area response is increased. The flow rate of the makeup gas mixture at NZ-NCNM is set to 7 ml min^{-1}. There are other advantages of using P10 (Ar/CH_4) as a makeup gas because it also eliminates a confounding effect of varying CO_2 concentration in the air samples on the determination of the N_2O concentration. Use of P10 also eliminates the need for CO_2 scrubbing (Wang, Wang, & Ling, 2010; Zheng et al., 2008).

A sample's peak area depends on the temperature of the ECD, and at the NZ-NCNM, 310 °C is used (Mosier & Mack, 1980; Wentworth & Freeman, 1973). Operating in the range of 330–350 °C has been recommended to help minimize cross-sensitivity of the detector to CO_2 (Butterbach-Bahl et al., 2016); however, this is not required at NZ-NCNM with use of the Ar/CH_4 makeup gas. Along with CO_2, the ECD is highly sensitive not only to N_2O, but other atmospheric gases, especially oxygen (O_2), water vapor, and halogenated hydrocarbons (chlorofluorocarbons [CFCs] and freons).

The main carrier gas flow rate is usually in the range of 30–40 ml min^{-1}. When using N_2 as the carrier gas, it should ideally be oxygen-free, "zero grade" purity N_2, which is further purified by passage through a chemical gas scrubber (e.g., Restek Corporation) to remove any residual oxygen and water vapor. Separation begins with the N_2 carrier gas "sweeping" the air sample via the double-cavity concentric injection needle, through a 1.5-mm (1/16th-inch) o.d. stainless steel transfer tube to Valve A, and then into the first of two 3-mm ($1/8^{th}$-inch) o.d. GC columns, each packed with screened (125–149 μm diam.), porous, resin beads (HayeSep D, a high-purity divinylbenzene polymer, Valco Instruments Company). Packed GC columns as opposed to capillary columns are still widely preferred for atmospheric N_2O analysis providing a robust and cost-effective solution. A stainless steel tube of 3.2 or 4.8 mm (1/8th or 3/16th inch) is recommended for the best balance of resolution, capacity, and operating cost (WMO, 2009).The GC configuration and run conditions are given in Table 2. During the "injection mode,: in the first half of a sample's run, the N_2 carrier gas is flushed through the Exetainer via Valve A at 2.5 times the ambient pressure, which injects the 6-ml gas sample onto a Hayesep D "pre-column" (Figure 3). The main components in an air sample—N_2, O_2, Ar, CO_2, CH_4, and N_2O—all pass rapidly through this pre-column into the main analytical column, whereas the passage of the slower moving components—water vapor, CFCs, and freons—is slowed.

The O_2 in the gas sample has a very short elution time on Hayesep D. To prevent it passing into the ECD and overloading that detector, Valve B, located at the posterior end of the analytical column, is switched to ensure that this large O_2 component is sent to waste. If this precaution is not taken, the response of the ECD to the more slowly eluting N_2O is compromised, making quantification more problematic (Zheng et al., 2008). Repeated exposure of the ECD to O_2 is also undesirable, as it is likely to shorten the life of the detector. The CH_4 in the air sample has a slightly longer elution time than O_2, but it can be quantified during this "injection mode" if the effluent gas is permitted to exit the analytical column via Valve B to an installed flame ionization detector (FID) rather than to waste (the waste line is not shown in the simplified Figures 3 and 4).

After about 4 min, during the last half of a sample's run time, both gas sampling valves are switched to a "backflush

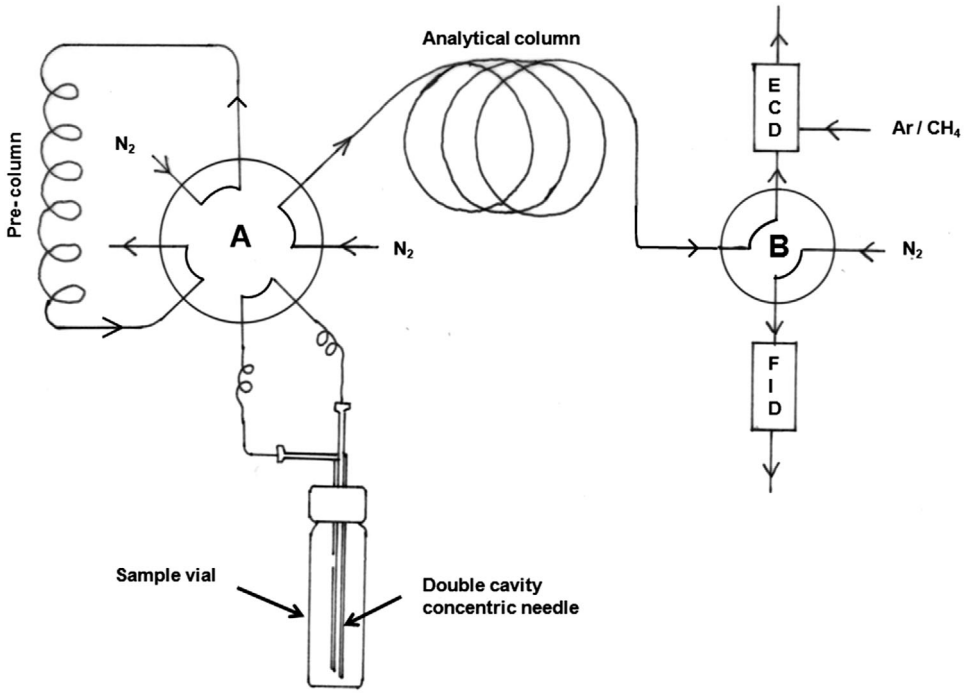

FIGURE 4 Simplified plumbing diagram, showing the gas sampling valves in the backflush mode as described in the text. The system shown is that used at New Zealand's National Centre for Nitrous Oxide Measurement (NZ-NCNM) and shows a second detector (flame ionization detector [FID]). Only the electron capture detector (ECD) is required for N_2O determination

mode" (Figure 4). During this period, the carrier gas flow through the "pre-column" is reversed, the slow-moving compounds are vented to waste, and the flow from the analytical column is rerouted through the ECD for quantification of N_2O. The N_2O elutes as a slightly non-Gaussian peak at 5.5 min after injection. At ~7.5 min, a separate command from the GC triggers the injection needle to move to the next Exetainer in the analytical sequence, isolating that next sample for injection at 8 min. This sequence is repeated, until all 220 samples and standards in a typical sample run have been analyzed.

5.4 | Multiple gases and analysis by GC–MS systems

The requirement for multi-greenhouse gas species analysis is commonplace, and a number of solutions have been developed. Hedley et al. (2006) describe a GC multi-gas system with FID-based detection of CH_4 and CO_2 after methanizer conversion along with N_2O by ECD. A 1-ml sample loop is used, with N_2 carrier gas, with Porapak QS 80/100 packed pre- and main-packed columns and multicomponent (CO_2, CH_4, and N_2O) gas standards. Ekeberg, Ogner, Fongen, Joner, and Wickstrøm (2004) used a quadrupole mass spectrometer (MS) in selective ion monitoring mode for the detection of these three greenhouse gases (CO_2, CH_4, and N_2O). Their setup used a 5-μl sample loop, helium carrier (2 ml min^{-1}), and CP-PoraPLOT Q-HT (Chrompak) main column. Detection limits and precision were similar to GC–ECD. One advantage of using the MS detector is its ability to quantify multiple components by single detector without gas-specific preprocessing.

6 | ANALYSIS BY OPTICAL AND OTHER DETECTORS

The GC analytical approach described in this paper, in combination with manual chamber sampling, will continue to have an important role in N_2O flux and emission factor data production in the foreseeable future, for a number of reasons including the low cost of field installation of chambers, the simplicity of deployment, the ability to measure at unpowered sites, the use of small gas sample volumes, the relative ease of transportation of sample sets between the field and laboratory, and the practicality of pooling analyses from multiple sites and organizations. Case by case, these advantages have to be weighed against disadvantages of lower accuracy of flux estimates for a site because the sampling is noncontinuous compared with automated systems. The spatial coverage is also small compared with spatially integrating measurements that can be obtained with micrometeorological systems. This can introduce significant errors where there is high areal variability in fluxes. Additionally, the higher cost of

labor for conducting the manual field sampling can be a consideration.

6.1 | Infrared laser-absorption spectroscopy

Beyond GC sample analysis, there is a new generation of spectroscopic technologies with high-finesse optical cavities that have resulted in a range of instruments capable of continuous measurement of N_2O concentrations in air. Such instruments include (a) quantum cascade laser (QCL) optical detectors, manufactured by Aerodyne Research (Nelson, McManus, Urbanski, Herndon, & Zahniser, 2004), (b) cavity ring down spectrometer with QCL (CRDS), manufactured by Picarro (Brannon et al., 2016; Christiansen, Outhwaite, & Smukler, 2015), (c) off-axis integrated cavity output spectroscopy with QCL (OA–ICOS), manufactured by Los Gatos Research (Brannon et al., 2016; Waldo et al., 2019). These instruments have the capacity to measure N_2O concentrations with a precision at the sub-nanomole-per-mole level, which is a performance improvement compared with conventional GC systems by at least an order of magnitude. The above manufacturers offer a range of different instrument configurations with the ability to measure other gas species simultaneously to N_2O, including CO_2, carbon monoxide (CO), or CH_4. Another technical advantage of these continuous analyzers is the linear response of the sensor, which reduces the number of required reference gases and the ability to accurately calibrate a larger concentration range.

These continuous high-precision analyzers are highly suitable for use with automated chamber systems and allow the operation of chambers with short closure periods. Mains power is usually required at the field site. It is important to follow specific calibration requirements for these analyzers, particularly with the composition of the calibration standards, their gas matrix, and with a calibration system design that avoids high standard gas consumption. Reference gases for optical analyzers have to be made from air mixtures that have ambient atmospheric proportions of main air components (N_2, O_2, and Ar), to avoid analytical artifacts such as pressure broadening (Brewer et al., 2019; Chen et al., 2010). Comparisons between conventional GC and automated QCL have been examined (Brümmer et al., 2017; Savage, Phillips, & Davidson, 2014) and show the value and superiority of newer generation optical analyzers compared with the conventional GC-based method. For more discussion of semi- or fully automated flux chamber systems, see Grace et al. (2020).

6.2 | Other detectors

Other detectors, also based on infrared absorption measurement, include Fourier-transform infrared (FTIR) spectroscopy analyzers, a precision optical technique that can measure over a broad range of infrared wavelengths. This also provides the ability to measure the three major greenhouse gases (CO_2, CH_4, and N_2O) and other species simultaneously. The FTIR technique described by Griffith et al. (2012) and Smale et al. (2019) is also well suited to coupling to automated chamber systems and has been successfully deployed on a range of agricultural systems such as pasture (Kelly, Phillips, & Baigent, 2008), mixed cropping (McDaniel et al., 2017), and sugarcane (*Saccharum* spp.; Denmead et al., 2010).

Photoacoustic trace-gas detection is a spectroscopic technique where specific infrared absorption is converted to an acoustic signal for measurement. Automated online chamber–photoacoustic analyzer systems have been deployed with the advantages of more rapid and frequent analysis (see also Grace et al., 2020). The technique has been discussed in summary by Butterbach-Bahl et al. (2016) and Iqbal, Castellano, and Parkin (2013), who note that the measurement may be of low precision and is susceptible to interference from other greenhouse gases through absorbance spectral overlaps, especially with water vapor and CO_2 that is abundant in chamber headspace air. Manufacturers of "Innova" multi-gas instruments (Lumascence Technologies) have carefully designed systems to apply corrections through measurement of potential interferent signals with specific bandpass filters. Independent correction schemes have also been developed (Tirol-Padre et al., 2014; van der Weerden, Clough, & Styles, 2013). Field study has shown that care is needed to get useful quantitative data (Debouk et al., 2018; Nicoloso et al., 2013) The instruments are also sensitive to environmental temperature (Debouk et al., 2018; Rosenstock et al., 2013) and other instrument drifts (Palzer, 2020). Providing that care is taken with deployment, analyzer costs are lower than with many other optical instruments, and portability is good compared with cavity laser spectroscopy.

A gas filter correlation instrument (Teledyne Analytical Instruments) has also been coupled with automated chambers in an evaluation of a low-power N_2O chamber system run on local wind and solar energy. For remote site operation, Warlo et al. (2018) showed that with the increasing portability of precision detectors, it is becoming increasingly practical to perform analyses on battery power in the field at remote sites. In addition to these commercial systems, development of lower cost sensors in the future is likely to provide practical and

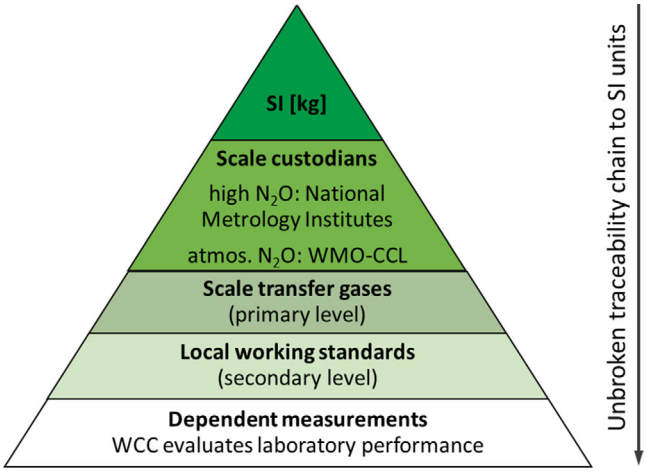

FIGURE 5 Traceability chain for measurements of atmospheric N_2O concentrations. (WMO-CCL: Central Calibration Laboratory, WCC: World Calibration Centre)

sensitive alternatives to conventional chromatographic or optical techniques. For example, methods do currently exist for the measurement of N_2O at microsites in the aqueous phase. Andersen, Kjær, and Revsbech (2001) describe a sensitive amperometric membrane microsensor (N_2O microsensor Unisense A/S), and this design has been used to examine profiles of N_2O production from thawing permafrost (Elberling, Christiansen, & Hansen, 2010).

7 | CALIBRATION

7.1 | Calibration gases for GC systems

All measurements should be calibrated against traceable reference standards linking to the SI system via fundamental gravimetric standards (Figure 5). For chamber analyses, gas mixtures with N_2O concentrations >370 nmol mol^{-1} are usually prepared gravimetrically by commercial manufacturers and suppliers of gas standards. The supplied standards have traceability through analysis against scale transfer reference gases that are prepared by a national metrology institute (e.g., National Institute of Standards and Technology [NIST, USA], National Physical Laboratory [NPL, UK], National Measurement Institute [NMI, Australia]) to link to the highest calibration hierarchy level referenced to mass standards maintained at the Bureau International des Poids et Mesures (BIPM). Calibration can be transferred on to working standards with suitable N_2O ranges. For example, at gas supplier BOC (Auckland and Christchurch, New Zealand, personal communication, 2020), gas mixtures (designated alpha or environmental grade) are prepared with specific N_2O concentrations by weighing pre-analyzed high-purity constituents into a cylinder, using high-capacity, high-accuracy balances. The composition of the mixture is calculated from the component masses, and the mixture accuracy can be ordered at ±1%. Although we found (Supplemental Section S3) that the calibration gas matrix did not affect the CV of the analyses, response differences have been observed between standards of N_2O in N_2 and N_2O in air ratio N_2/O_2, and care should be taken with the gas matrix. An air matrix gas made from ultrapure synthetic air components was found to have less remaining N_2O traces than purified natural air and is thus preferred for the highest accuracy (Brewer et al., 2019). However, the quality of both synthetic and purified natural matrix gases may vary between gas providers, whereas some calibration mixtures may only be available as N_2O diluted in N_2 so that a generalized recommendation may not be applicable to all laboratories. Laboratories measuring N_2O should be aware of potential calibration artefacts that are dependent on the method by which their calibration gases were made. It is therefore recommended for the laboratories to be aware of the manufacturing process of their calibration gases and to allow some overlap between standards when calibration gas cylinders are due for replacement. All mixtures used here are verified by analyzing the N_2O concentrations of the produced gases against higher level standards prepared by a national metrological institute using GC and/or FTIR. The Primary Gravimetric Standard production facilities in New Zealand are accredited to ISO Guide 34, by the National Association of Testing Authorities (NATA, Australia) and International Accreditation New Zealand (IANZ, New Zealand), producing documented standards. The manufacture is in compliance with ISO Method 6142 (ISO, 2015). For background on traceability, the reader is also referred to ISO Method 14167 (ISO, 2018).

At close to ambient concentrations (~300 nmol mol^{-1}), research gas standards of much higher accuracy are available for N_2O < 370 nmol mol^{-1} (nanomoles per mole of dry air) calibrated on the World Meteorological Organization (WMO) mole fraction scale. Prior to 2006, laboratories were assessed on the WMO-X2000 scale, and after that on the WMO-X2006A scale (Hall, Dutton, & Elkins, 2007), which covers the range of 261–371 nmol mol^{-1}. Standards prior to 2000 were prepared at Scripps Institute of Oceanography (SIO) and henceforth at the WMO Central Calibration Laboratory (CCL, NOAA). Regular calibration of the working standards against the scale transfer gases ensures the stability of the local calibration scheme. Primary standard gas cylinders ideally have a long life and are mainly kept for scale transfer to secondary standards by gas analysis and onward transfer of the calibration scale to laboratory working standards at individual laboratories. Thus, the traceable hierarchy of gas standards

(Figure 5) exists globally with a target for compatibility (0.1 nmol mol^{-1}), which is regularly checked by traveling standards. The scale is managed through the World Calibration Centre WCC-N_2O hosted by the Karlsruhe Institute of Technology (KIT), Institute of Meteorology and Climate Research (IMK-IFU), Garmisch-Partenkirchen, Germany. The integrity of dependent measurements is independently tested and internationally compared through the WCC-N_2O. This process for N_2O is described in detail by Zellweger et al. (2019). Atmospheric research laboratories, including the NIWA Gas Laboratory in Wellington, produce their own working standards and quantify them on the current WMO scale by means of the scale transfer gases supplied by the CCL.

7.2 | GC run calibration

Each GC "run" should be calibrated by including standards ("reference" gases) that cover the concentration range encountered in the samples. The system performance can be further examined by including ambient reference "check" samples throughout the run. By way of example, at the NZ-NCNM, calibration of each GC system involves 10 standards. There are nine alpha-grade standards in G-sized cylinders that were composed of a mixture of compressed N_2O and N_2 gas. These alpha-grade, synthetic gas mixtures were prepared gravimetrically by BOC as described and were subsequently analyzed to ensure local laboratory results were well within their guaranteed 95% confidence interval (typically ±1–2% of the N_2O concentration).

Of the 10 standards used at NZ-NCNM, one is a synthetic standard made of pure N_2 gas, whose N_2O concentration is zero. The main purpose of this standard is to account for the very small quantity of N_2O from background laboratory air inevitably associated with the injection process itself, resulting in a very small positive N_2O peak area for that "blank" N_2O standard (see Figure 6). The next standard has a sub-ambient N_2O concentration of 200 (± 10) nmol mol^{-1}. In addition, a whole air standard is used, which was collected outside the NIWA laboratory at Greta Point, Wellington, in August 2009 in clean conditions. A mean N_2O concentration for five samples of this bottled air from Greta Point was 321.3 (± 0.3 nmol mol^{-1}), the error being twice the standard deviation for 95% confidence interval (Gordon Brailsford, personal communication, 2009). The remaining seven synthetic standards had super-ambient N_2O concentrations of 500 (± 10), 1,000 (± 10), 2,000 (± 20), 5,000 (± 100), 10,000 (± 200), 20,000 (± 400), and 50,000 (± 1000) nmol mol^{-1}.

Interlaboratory comparisons are informative and recommended. This can involve the exchange of samples of a single concentration, as well as standards. As a warning, for example, after five western Canadian laboratories exchanged air samples of a single concentration, as well as a "reference" standard, reported means for the air sample ranged from 650 to 1,000 nmol mol^{-1} (Lemke et al., 2002). Using results for the reference standard, statistical analysis suggested a major source of the interlaboratory variability could be attributed to the standards, with different laboratories having had different commercial suppliers, evidently of variable quality.

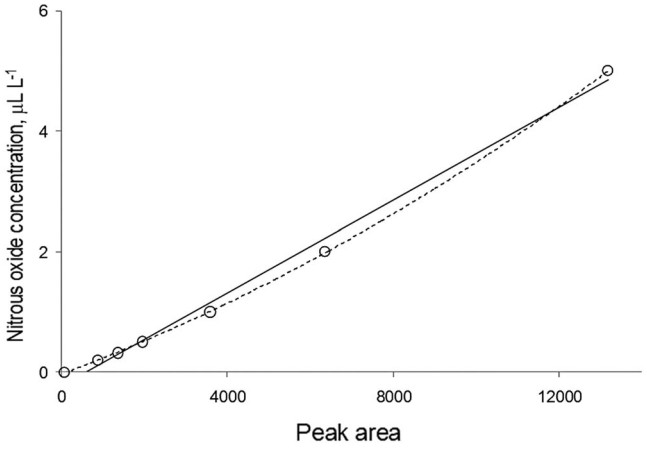

FIGURE 6 The relation between peak area and N_2O concentration, determined by calibrating a gas chromatograph (GC4) at New Zealand's National Centre for Nitrous Oxide Measurement (NZ-NCNM) on 30 Nov. 2011. On the basis of two regressions compared by an F statistic, a linear relation (solid) did not fit these data as closely as a quadratic curve (dashed, $p < .001$, N_2O concentration [µl L^{-1}] = $-0.036 + 2.569 \times 10^{-4}$ peak area + 9.544×10^{-9} peak area^2)

7.3 | Processing GC data

There is a variety of proprietary or system specific (e.g., Agilent Chemstation) as well as open-source (e.g., Open Chrom) chromatography software acquisition and analysis systems for use with ECD, which we will not go into in this paper. To optimize the precision and accuracy of results, the GC analysis procedure should be automated. At the NZ-NCNM, the GC system software controls valve switching (Figures 3 and 4), records and integrates analogue signals from the ECD and FID at 1 Hz, and displays chromatograms on the controlling computer's screen. After analysis of each batch of samples, the post-run chromatograms are scrutinized, and baseline correction and adjustment of "integration windows" are applied if necessary. An integrated results file can then be exported to a worksheet (e.g., Excel) for further post-run processing.

Post-run processing begins with the standards, determining a relationship between peak area and N_2O concen-

tration for the GC system. Although all standards should be included in each GC run, a selection may be more appropriate for determining the relationship, depending on peak areas of the samples. For example, to calibrate a GC, seven standards were selected, including N_2O concentrations of 0, 200, 321.3, 500, 1,000, 2,000, and 5,000 nmol mol^{-1} (Figure 6). This GC run included one sample with an N_2O concentration <321.3 nmol mol^{-1} (the minimum with 320 nmol mol^{-1}), 43 between 321.3 and 500 nmol mol^{-1}, 25 between 500 and 1,000 nmol mol^{-1}, 15 between 1,000 and 2,000 nmol mol^{-1}, and six between 2,000 and 5,000 nmol mol^{-1}, with a maximum value of 3,200 nmol mol^{-1}. Linear and quadratic (second-order polynomial curve) models were fitted to these data by regression methods. Using an *F* statistic (Snedecor & Cochran, 1991), the two (nested) models were compared and the quadratic model was a significantly better fit than the linear model and, in this case, is recommended for GC calibration.

In fact, the ECD GC detector is an inherently nonlinear detector, and this is important to take account of, especially over large concentration ranges. A method using addition of CO_2 makeup gas has been suggested by Zhang, Mu, Fang, and Liu (2013) as a way to significantly improve linearity of the ECD response to N_2O. For future consideration, modern optical detectors have advantages of good linearity in response compared with the ECD (Lebegue et al., 2016).

There are basic statistics that can be calculated to quantify GC performance. To illustrate some principles following Ellison, Barwick, and Farrant (2009), a *repeatability standard deviation*, SD_r, can be calculated from results obtained using one method and a set of replicate air samples from the same source, under the same conditions—operator, GC system, and laboratory. This statistic, quantifying within-laboratory variability by a square root of the within laboratory variance, SD_{wi}^2, may be written:

$$SD_r = \sqrt{SD_{wi}^2} \quad (1)$$

A *reproducibility standard deviation*, SD_R, applies to results using the same method, and sets of replicate air samples under different conditions (different operators, GC systems, and laboratories). This statistic quantifies total variation, calculated by combining the between-laboratory variance (SD_{be}^2), the sample–laboratory interaction (if it exists), and SD_{wi}, and it may be written:

$$SD_R = \sqrt{SD_{be}^2 + SD_{wi}^2} \quad (2)$$

Repeatability, *r*, is the value below which an absolute difference between two single test results—obtained with the same method on sets of replicate air samples under the same conditions (operator, GC system, laboratory, and a short interval of time)—may be expected to lie. This may be written:

$$r = t\sqrt{2}SD_r \quad (3)$$

where *t* is the Student *t*, two-tailed value, for $n - 1$ (*n*, number of replicates) for a given confidence, usually 95%. Finally, reproducibility, *R*, is the value below which an absolute difference between two single test results, obtained with the same method, on sets of replicate air samples under different conditions (operator, GC system, laboratory, and/or different times) may be expected to lie. This may be written:

$$R = t\sqrt{2}SD_R \quad (4)$$

In the Supplemental Information, we expand an example of the uncertainty in analyses of sample batches (Supplemental Table S2-1).

8 | RELATING NITROUS OXIDE SAMPLE ANALYSES TO NITROUS OXIDE FLUXES

For measurements of N_2O fluxes by the chamber method, key variables include the height of the soil chamber head space, the interval between gas samples taken from the head space, and precision (SD_{N2O}) of the GC system. The GC system's precision will quantify the variability of measurements for a set of samples, each having the same N_2O concentration. In essence, the smaller the variability, the greater the signal-to-noise ratio of a GC system.

Repeatability in gas measurement should not limit flux detection capability. It is recommended that error in flux estimate due solely to laboratory repeatability should be at least an order of magnitude smaller than fluxes being measured. For a full discussion of the impact of analytical precision on overall error in the calculated chamber fluxes, we refer the reader to the Section 3.1 in Venterea et al. (2020).

9 | CONCLUSIONS

From sample collection in the field to laboratory GC analyses, we have recommended best practices to maximize accuracy of measurement. The sampling procedures should include the following:

- the use of clean, nonreactive, pre-evacuated sampling vials,

- overpressure of collected gases to give best assurance of sample integrity,
- gas drying at the point of collection, and
- minimized storage times and co-storage of some calibration gas vials, especially where longer storage is likely.

Gas analysis should include the following best practices:

- Traceable calibration gases should be analyzed along with the samples.
- The unknowns should fall within the concentration range of measured standards.
- With ECD, nonlinear calibration is recommended for most accurate determination of gas concentration.
- Repeat analyses should be included to allow for determination of precision of the GC gas measurements.
- The sample error directly influences the overall uncertainty of the resulting final flux estimates and should not be an overriding limitation of uncertainty in flux.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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